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Phytochemical Evaluation and Cytotoxic Activities of Stem Bark and Leaf Extracts of *Mesua assamica*

(Penilaian Fitokimia dan Aktiviti Sitotoksik Ekstrak Kulit Batang dan Daun *Mesua assamica*)

Hazlina Ahmad Hassali[1,*], Chan Gomathi[2], Wan Hamirul Wan Bahrin[1], Zainah Adam[1], Daryl Jesus Arapoc[1], Rosniza Razali[1], Azfar HanifAbd Aziz[1] & Khalijah Awang[2]

[1]*Medical Technology Division, Malaysian Nuclear Agency, 43000 Kajang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Chemistry, Faculty of Science, University Malaya, 59100 Kuala Lumpur, Federal Territory, Malaysia*

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ABSTRACT

Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. The *Mesua* (Calophyllaceae) has been known to produce various new chemical compounds of medicinal values. Some *Mesua* species have yielded new potential anticancer agents that are important to the pharmaceutical industry. In this research, phytochemical constituents, antioxidant and cytotoxic activities of different solvent extracts of *Mesua assamica* stem bark and leaves were evaluated. The stem bark and leaves of *M. assamica* were successively extracted in hexane, ethyl acetate and methanol. Qualitative phytochemical analysis showed that most of the *M. assamica* extracts consist of important phytochemicals, namely, anthraquinones, terpenoids, flavonoids, saponins, tannins, phlobatannins, alkaloids, cardiac glycosides, glycosides, decreasing sugars, steroids, lipids, phenols, coumarins, carbohydrates, proteins, and betacyanin indicating its potential for medicinal use. Quantitative determination of total phenolics, total flavonoids, and *in vitro* antioxidant activities through DPPH assay of *M. assamica* extracts have been achieved by utilizing colorimetric methods. *In vitro* cytotoxic evaluation through MTT assay induction against human breast MCF-7 cancer cell lines exhibited that hexane extracts were found to have IC_{50} value below 30 µg/mL and conferred effectiveness in inducing cell death MCF-7. The diversity of phytochemicals present suggests that the stem bark and leaves of *M. assamica* could serve as a supply of potentially valuable medications. Exploiting the plant's pharmacological qualities will necessitate more study on the isolation, purification, and identification of bioactive components.

Keywords: Antioxidant; cytotoxic; *Mesua assamica*; MTT assay; phytochemicals

ABSTRAK

Produk semula jadi dan bahan terbitannya telah dikenal pasti sebagai sumber agen terapi yang tidak ternilai. Spesies *Mesua* (Calophyllaceae) diketahui dapat menghasilkan pelbagai sebatian kimia baharu yang mempunyai nilai perubatan. Terdapat spesies *Mesua* yang berpotensi sebagai agen antikanser dan penting dalam industri farmaseutikal. Dalam kajian ini, unsur fitokimia, antioksidan dan sitotoksik dalam ekstrak Penaga Bayan (*Mesua assamica*) dan daunnya dinilai. Kayu dan daun *M. assamica* diekstrak secara berturutan dalam pelarut heksana, etil asetat dan metanol. Analisis fitokimia secara kualitatif mendedahkan bahawa sebahagian besar ekstrak *M. assamica* terdiri daripada fitokimia penting, iaitu antrakuinon, terpenoid, flavonoid, saponin, tanin, phlobatannin, alkaloid, glikosida jantung, glikosida, menurunkan paras gula, steroid, lipid, fenol, kumarin, karbohidrat, protein, dan betasianin berpotensi sebagai kegunaan dalam perubatan. Penentuan jumlah fenol, jumlah flavonoid dan aktiviti antioksidan *in vitro* melalui ujian DPPH bagi ekstrak *M. assamica* secara kuantitatif telah tercapai dengan kaedah kolorimetrik. Penilaian sitotoksik *in vitro* melalui induksi ujian MTT terhadap saluran sel kanser MCF-7 payudara manusia menunjukkan bahawa ekstrak heksana didapati mempunyai nilai IC_{50} di bawah 30 µg/mL dan memberikan keberkesanan dalam mendorong kematian sel MCF-7. Kepelbagaian fitokimia yang hadir menunjukkan bahawa kayu dan daun *M. assamica* boleh berfungsi sebagai bekalan ubat-ubatan yang berpotensi dan berharga. Kepelbagaian fitokimia yang ada menunjukkan bahawa

kayu dan daun *M. assamica* mampu berfungsi sebagai sumber perubatan yang berpotensi. Mengeksploitasi kualiti farmakologi tumbuhan akan memerlukan lebih banyak kajian tentang pengasingan, penulenan dan pengenalpastian komponen bioaktif.

Kata kunci: Antioksidan; fitokimia; *Mesua assamica*; sitotoksik; ujian MTT

Introduction

Natural products, particularly plants, have been utilized for healing for as ancient as medicine has been (Adhikari et al. 2022; Dutta et al. 2020). Scientists have long been encouraged to document their observations because of Malaysia's tropical flora wealth, which results in the usage of medicinal plants as therapeutic and curative representatives for diverse nutrition. According to epidemiological research, the raised consumption of plant-based, antioxidant-rich foods, such as fruits, whole grains, nuts, and vegetables has been linked to a lower risk of various chronic diseases. According to current estimates, about 80% of the world's population receives health treatment from traditional medicine (Baruah et al. 2021; Satheesh et al. 2013). When applied carefully, medicinal plants comprise of therapeutic bioactive chemicals that have demonstrated to be effective as essential or additional remedies (Chang et al. 2008). Bioactive compounds, widely recognized as phytochemicals, refer to every naturally occurring chemical present in plants. Non-nutritive plant compounds with disease-preventive or protective properties are known as phytochemicals. More than thousand known phytochemicals are classified into main compounds based on biosynthetic origin, solubility properties, and functional groups, namely phenolic compounds, terpenoids, organic acids, and nitrogen compounds such as alkaloids sugars and their derivatives as well as macromolecules (Harbone 1998). Phytochemicals can be employed as medications in their natural state, as basic materials for partial drug synthesis, or as models for the development of new pharmaceuticals (Ibrahim 2004).

According to a scientific study, antioxidants have a significant role in health protection, antioxidant helps in reducing the danger of chronic illnesses like cancer and heart disease. Antioxidant activities can be determined by different types of assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH) essentials, decreasing strength assay and ß-carotene method. DPPH is a commonly used method for evaluating antioxidant activity and testing the ability of substances to serve as freeradical scavengers or hydrogen donors (Shekahar & Gopal Anju 2014).

Malaysia is blessed with diverse varieties of medicinal plants as source of potential to produce therapeutic effects and may hold the key to developing new anti-cancer drugs. *Mesua* is a big genus with over 48 species of plants which are extensively scattered around plentiful equatorial countries, for example India, Burma, Thailand, Indochina and New Guinea (Ee et al. 2005). Previous research showed that *Mesua* exhibits various pharmacological and biological activities such as antioxidant, antiacetylcholinesterase (AChE), and larvicidal activities, hepatoprotective, antibacterial, antiarthritic, immunomodulatory, neuromodulator, antiproliferative, and antiviral activities (Ee et al. 2012). *Mesua assamica* is an important tropical medicinal plant to be discovered for its medicinal properties. *M. assamica* comes from the family of Calophyllaceae and usually known as *'SiaNahar'* (in Assamese). This plant is widely available at the foothills of the Himalayas in the North Lakhimpur subdivision of Assam, India. *Kayea assamica* is the other genus name for *Mesua assamica* (Phukan et al. 2017). It is a tall evergreen, moderately growing plant in common habitat. *M. assamica* commonly found in Assam, India; Kachin, Myanmar; Kedah, Kelantan, Terengganu, Pahang, Negeri Sembilan and Johor; Peninsular Malaysia and Borneo. The plant has been accounted from Dullung and Kakoi Forest Reserves of Lakhimpur region of Assam in India. Its bark has colour of light brownish grey, while its leaf is ovate or elliptic-lanceolate in shape. Furthermore, its flower is white in colour and its seed and fruit is depressed and globose in shape (Gogoi 2017).

Although there are a number of scientific reports on the pharmacological activities on other species of this plant, there are limited studies about the phytochemicals and cytotoxicity works associated with *M. assamica* species. Furthermore, evaluating the presence of phytochemical constituents is the main objective of this study. It also seeks to use the DPPH assay to measure total phenolics, total flavonoids, and *in vitro* antioxidant properties. as well as to assess the cytotoxic activities using MTT assay in distinct solvent extracts of *M. assamica* stem bark and leaves. The findings of this study

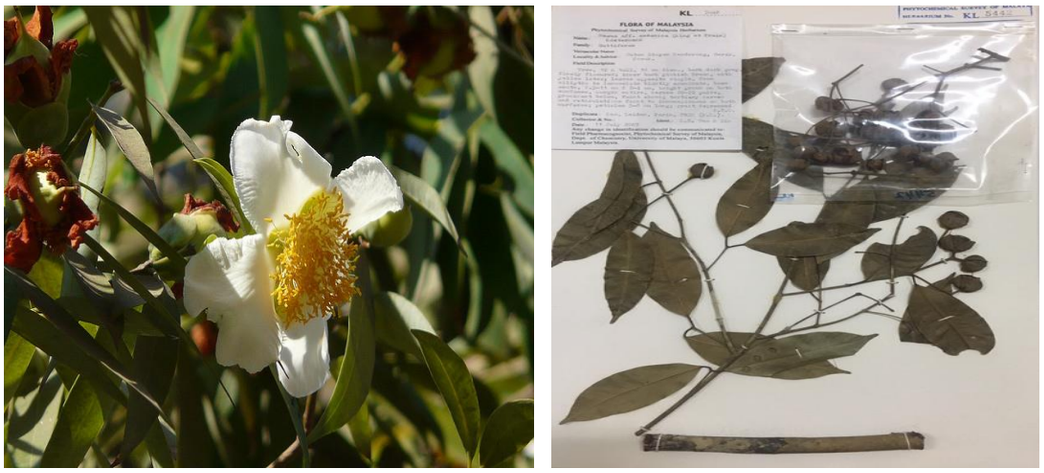

FIGURE 1. *Mesua assamica* plant

are preliminary data that should be further investigated for use in health products, the food sector, and medical and pharmaceutical applications.

Materials and Methods

Plant Materials

Plants of *M. assamica* were collected from Hutan Simpan Kenderong, Gerik, Perak in July 2007. The Herbarium of the Laboratory of Natural Products, University of Malaya, Kuala Lumpur, Malaysia, received a voucher specimen (KL 5442). The stem bark and leaves of *M. assamica* have been separated carefully, cleansed, and air dried before being oven dried for 24 h at 40 °C. *M. assamica* dry leaves were pulverised into a coarse powder (Figure 1).

Preparation of Extracts

The powdered stem bark and leaves of *M. assamica* have been subordinated to sequential solvent extraction with hexane, ethyl acetate and methanol. This process involved maceration with each solvent for three cycles; each cycle involving one day soaking and shaking using wrist shaker at room temperature. The next solvent has been inserted to the sediment after filtration, and the extraction was carried out for the next 3 days in a similar way. To get concentrated hexane, ethyl acetate, and methanol extracts, the extracts have been filtered and intensified using a rotary evaporator under reduced pressure at 40 °C (Buchii, Switzerland). The extracts have been preserved in vacuum oven for further usage.

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening has been utilized to assess the partnership of secondary metabolites in hexane, ethyl acetate and methanol extracts of *M. assamica* utilizing standard procedures and methods with just minor modifications to be established (Ayoola et al. 2008; De et al. 2010; Edeoga et al. 2005; Tiwari et al. 2011). The following tests have been conducted to identify the primary chemical groupings found in nature, such as anthraquinones, terpenoids, steroids, saponins, alkaloids, carbohydrates, cardiac glycosides, glycosides, reducing sugars, flavonoids, lipids, coumarins, phlobatannins, tannins, proteins, phenols and betacyanin in all extracts:

Anthraquinones

A portion of the extract (0.5 g) has been heated in 10 mL of H_2S0_4 and then filtered while it was still hot. Five mL chloroform was added to the filtrate that has been shaken. The methanol layer was pipetted into a new test tube, which was then filled with 1 mL of dilute ammonia. As a result of the solution, colour changes were noted.

Terpenoids

Two mL of chloroform has been inserted to each 0.5 g extract. Sulphuric acid concentrated H_2S0_4 (3 mL) has been added with caution to shape a layer. The participation of terpenoids is indicated by the interface's reddish-brown colour.

Flavonoids
One g of each extract has been cooked in 10 mL of distilled water for 5 min, then it was filtered while it was hot. Few drops of 20% sodium hydroxide solution have been inserted to 1 mL of cooled filtrate, followed by adding of dilute hydrochloric acid. The occurrence of flavonoids was confirmed by yellow solution with NaOH that turned colorless when treated with dilute HCl.

Saponins
In a test tube, 5 mL distilled water has been added to 0.5 g extract. A persistent, continuous foam was noted after strong agitation of the solution. The foam was mixed with three drops of olive oil and vigorously stirred before being tested for the production of an emulsion.

Tannins
In a test tube, about 0.5 g of the extract has been heated in 10 mL of water and then filtered. Few drops of 0.1% ferric chloride have been inserted. The colour was spotted for brownish green or a blue-black.

Phlobatannins
Each plant sample's aqueous extract (1 drop) was boiled mixed with 1 percent aqueous hydrochloric acid. The occurrence of phlobatannins has been noted by the formation of a crimson precipitate.

Alkaloids
Two mL of each extract, 1% of hydrochloric acid and 6 drops of Dragendorffs reagent were added. An orange precipitate noted the occurrence of alkaloids in the sample.

Cardiac Glycosides
Five mL of extract has been mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. With caution, the above combination was poured to 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture. A brown ring would appear in the sample if cardiac glycoside was present, indicating the presence of the cardiac glycoside constituent.

Glycosides
Modified Borntrager's Test: Each extract (1 drop) was hydrolysed with dilute HCl. After being treated with Ferric Chloride solution, the extracts have been submerged in boiling water for 5 min. After cooling, an equal amount of benzene has been used to extract the mixture. An ammonia solution was used to separate the benzene layer and cure it. The presence of anthranol glycosides in the ammonical layer indicates the creation of a rose-pink tint.

Reducing sugars
Fehling's test: Each extract (1 drop) was dissolved in 5 mL of distilled water and filtered. The filtrate was then boiled for a few minutes with 5 to 8 drops of Fehling's solution A and B. The presence of reducing sugars is indicated by an orange or brick red colour.

Steroids
Two mL acetic anhydride was mixed with 2 mL H2S04 in a 0.5 g extract of each sample. The presence of steroids was identified by a colour change from violet to blue or green in the same samples.

Lipids
Following two drops of extract, there should be oil on the filter paper.

Phenols
Each extract (1 drop) was mixed with 3 mL of 5% $FeCl_3$ and 5 drops of $K_3Fe\ (CN)_6$. Formation of dark green precipitate indicated the presence of phenols.

Coumarins
Three mL of 10% NaOH was added to 2 mL of plant extract. Formation of yellow colour indicates the presence of coumarins.

Carbohydrates
Each extract (1 drop) was dissolved in 5 mL distilled water and was filtered. The filtrates were hydrolysed with dilute HCl, neutralized with 10% of NaOH and heated with Fehling's solution A and B. The presence of decreasing sugars is reflected on the formation of red precipitate.

Proteins
A few drops of strong Nitric acid were added to each extract (1 drop). The existence of proteins is clearly reflected by the figuration of a yellow.

Betacyanin
Each extract (1 drop) was mixed with 10 mL of distilled water and placed in a 125 mL flask. Then, while swirling the flask, 2 mL of 2N NaOH was added to the flask drop-wise. Appearance of yellow color indicates presence of betacyanins.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING ACTIVITY PREPARATION OF STOCK SAMPLE

Each of the *M. assamica* extracts (100 mg) were weighed and kept in microcentrifuge tube. The extracts were then dissolved in 1 mL of 100% DMSO to form a concentration of 100 mg/mL stock solution.

TOTAL PHENOLIC CONTENT (TPC)

Total phenolic content was analyzed using the Folin-ciocalteu reagent (Yang et al. 2011). Crude extract (10 µL) was taken out from 100 mg/mL stock and diluted with 990 µL ethanol to form concentration of 1 mg/mL working solution. This was applied to all extracts. Then, 100 µL of 1 mg/mL of each crude extract diluted in ethanol was loaded into Falcon tube. Next, 4.5 mL of distilled water was added into the extract. The extract was then mixed with 100 µL of 2N Folin-ciocalteu reagent and shaken for 3 min. After that, 200 µL of 2% sodium carbonate solution was added and the mixture was allowed to incubate at room temperature for 3 h. A standard calibration curve of gallic acid in the range of 0, 62.5, 125, 250, 500, and 1000 µg/mL was prepared in the same manner. After 3 h, 200 µL of the mixture was transfer (in triplicate) into a 96-wells plate. The absorbance was taken at 760 nm utilizing EnSpire® Multimode Plate Reader (Perkin Elmer, USA). The phenolic content was expressed as µg gallic acid equivalence (GAE) per mg extract.

TOTAL FLAVONOID CONTENT (TFC)

The total flavonoid content was calculated using the aluminum chloride ($AlCl_3$) solution (Handore et al. 2018; Yang et al. 2011). Crude extract (10 µL) was taken out from 100 mg/mL stock and diluted with 990 µL distilled water to form concentration of 1 mg/mL working solution. This was applied to all extracts. 100 µL of 1 mg/mL of each crude extract diluted in distilled water was loaded (in triplicate) into 96-well plate. 100 µL of 2% of $AlCl_3$ solution was added into each well with 100 µL sample. Quercetin was prepared as standard calibration curve for the assay in 0, 5, 10, 50, 100, and 200 µg/mL concentration. The plate was incubated for 30 min at room temperature and the absorbance at 435 nm was read by utilizing EnSpire® Multimode Plate Reader (Perkin Elmer, USA). The flavonoids content was expressed as µg Quercetin equivalence (QE) per gram of extract.

DPPH SCAVENGING ACTIVITY

The free radical scavenging activity of *M. assamica* extracts was determined using the method described by Brand-Williams et al. (1995), with minor modifications. The free radical scavenging activity of sample extracts was measured using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. The stock was produced at a concentration of 100 mg/mL and diluted in 100 percent DMSO. 10 µL of crude extract was taken from the stock and diluted in 990 µL of ethanol to form concentration of 1 mg/mL working solution. 100 µL of ethanol was added into all 96-wells plate. 100 µL of sample were dispensed into wells of first row and were made in triplicate. Dilution with ethanol across plate was done to form concentration of 250, 125, 62.5, 31.25, 15.625, 7.81, 3.91, and 0 µg/mL. 100 µL of 0.2 mM DPPH dissolved in ethanol was inserted into all wells. The plate was incubated for 30 min at room temperature in dark condition to complete the reaction. This was followed by reading of absorbance at 517 nm utilizing EnSpire® Multimode Plate Reader (Perkin Elmer, USA). Ascorbic acid was anticipated as standard. The % of inhibition against concentration was calculated for each concentration and the graphs were plotted. The following equation was used to count the DPPH radical concentration.

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

where $A_{control}$ and A_{sample} are the absorbance values of the control and test sample, respectively. The IC_{50} (concentration providing 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract. From this curve, radical scavenging activity was expressed in IC_{50} value (µg/mL).

ANTI-PROLIFERATIVE ACTIVITY ASSAYS

Cell culture

Bio-Diagnostics, Malaysia, provided the chemicals and reagents used in the cell culture investigations. American Type Culture Collection provided the MCF-7 human breast cancer cell line and the human adipocyte cell line used in the study (ATCC). The cells have been cultured in Dulbecco's Modified Eagle Medium (DMEM) accomplished with 10% Fetal Bovine Serum (v/v) (FBS), 1 % (v/v) antibiotic solution (50000 IU/L, 1 % (v/v) Glutamax, and penicillin-streptomycin) at 37 °C humidified with 5% CO_2.

Antiproliferative activity assay

A standard MTT assay (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) was used to determine

the inhibition of growth of the cultured cancer cells line. The assay detects the decrease of yellow MTT dye by metabolically active cells, which is caused in part by the action of viable cell's mitochondrial dehydrogenase on purple formazan crystals (Wiratchanee et al. 2010). Confluence cells in 75 cm^3 culture flasks were harvested and stock of cells at concentration of 1.5×10^5 cells/mL was prepared. From this stock, 100 µL of cell suspension was seeded into sterile 96-well plates and 100 µL of complete culture medium was added into each well. Cells were left for 12 h at 37 °C to allow attachment prior to test. The next day, the media in each single well was softly aspirated and 100 µL of new complete culture medium was added. The cells were then treated with *M. assamica* extracts at various concentration (100 µL each well). Each extract yielded eight concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.8125, and 3.9 µg/mL). Cells were incubated for 72 h at 37 °C. Following the required incubation period, 20 µL of 5 mg/mL of MTT solution dissolved in phosphate buffer pH 7.4 was inserted into each provided well and kept for 4 h at 37 °C. Following that, each well's medium was gently aspirated and 100 µL of dimethylsulfoxide (DMSO) has been added into each well to melt the formazan crystals. The plates have been vibrated for 5 s before being tested for absorbance using EnSpire® Multimode Plate Reader (Perkin Elmer, USA) at 570 nm and The IC_{50} values (concentrations that inhibit cell growth by 50%) were taken down. To do the statistical analysis, all of the experiments were done in triplicate and three times.

Results

Percentage yield of extracts

Tables 1 and 2 showed the results of weight and percentage yield of *M. assamica* crude extracts using different solvents from 500 g of leaves and bark, respectively. The results showed that methanol extract produced the highest yield with 50.22 g (10.04%) followed by ethyl acetate extract with 7.53 g (1.50%) and the least was hexane extract with 5.84 g (1.17%) in leaves samples of *M. assamica.* Meanwhile for bark samples of *M. assamica*, the results showed that methanol extract produced the highest yield with 61.66 g (12.33%) followed by hexane extract with 13.8 g (2.76%) and the least was ethyl acetate extract with 9.46 g (1.89%). These results suggested that methanol showed to be a better solvent for extraction when compared with the remaining solvent used in the extraction.

TABLE 1. Percent yield of *M. assamica* leaves extracts in organic solvent

Extract	Weight of the powdered sample (g)	Weights of sample extract (g)	Percent (%) yield
Hexane	500 g	5.84 g	1.17 %
Ethyl acetate	500 g	7.53 g	1.50 %
Methanol	500 g	50.22 g	10.04 %

TABLE 2. Percent yield *M. assamica* bark extracts in organic solvent

Extract	Weight of the powdered sample (g)	Weights of sample extract (g)	Percent (%) yield
Hexane	500 g	13.80 g	2.76 %
Ethyl acetate	500 g	9.46 g	1.89 %
Methanol	500 g	61.66 g	12.33 %

PRELIMINARY PHYTOCHEMICAL SCREENING

The qualitative phytochemical screening results of *M. assamica* extracts are summarized in Tables 3 and 4. Based on the results, all extracts exhibited significant indication about the presence of important metabolites. Phytochemical screening tests in *M. assamica* leaf extracts showed the presence of flavonoids, alkaloids, cardiac glycoside, reducing sugar, steroids, triterpenes, lipids, phenols, coumarins, carbohydrates, proteins, and betacyanin.

Based on Table 4, In all *M. assamica* bark extracts, phytochemical screening showed the occurrence of anthraquinones, terpenoids, flavonoids, alkaloids, cardiac glycosides, reducing sugar, steroids, triterpenes, lipids, phenols, coumarins, carbohydrates, and proteins.

TABLE 3. Qualitative phytochemical analysis of *M. assamica* leaf exracts

Secondary Metabolites	Hexane	Ethyl Acetate	Methanol
Antraquinones	-	+	+
Terpenoids	+	-	+
Flavonoids	+	+	+
Saponins	-	-	-
Tannins	-	-	+
Phlobatannins	-	-	+
Alkaloids	+	+	+
Cardiac Glycosides	+	+	+
Glycosides	-	-	-
Reducing sugar	+	+	+
Steroids	+	+	+
Triterpenes	+	+	+
Lipids	+	+	+
Phenols	+	+	+
Coumarins	+	+	+
Carbohydrates	+	+	+
Proteins	+	+	+
Amino acids	-	-	-
Carotenoids	-	-	-
Anthocyanins	-	-	-
Anthocyanin and Betacyanin	+	+	+

(+), Presence, (-), Absence

TABLE 4. Qualitative phytochemical analysis of *M. assamica* bark extracts

Secondary metabolites	Hexane	Ethyl Acetate	Methanol
Anthraquinones	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Saponins	-	-	-
Tannins	-	-	+
Phlobatannins	-	+	+
Alkaloids	+	+	+
Cardiac Glycosides	+	+	+
Glycosides	-	-	-
Reducing sugar	+	+	+
Steroids	+	+	+
Triterpenes	+	+	+
Lipids	+	+	+
Phenols	+	+	+
Coumarins	+	+	+
Carbohydrates	+	+	+
Proteins	+	+	+
Amino acids	-	-	-
Carotenoids	-	-	+
Anthocyanins	-	-	+
Anthocyanin and Betacyanin	+	+	-

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Total phenolic content of M. assamica extracts

The phenolic content has been expressed as µg gallic acid equivalence (GAE) per mg extract by reference to standard curve (Figure 2). Based on total phenolic content shown in Table 5, methanol bark extract had the highest total phenolic content of 3.1170 ± 0.0166 µg GAE/g, followed by leaf methanol, bark ethyl acetate, bark hexane, leaf ethyl acetate and leaf hexane extracts which were 3.0028 ± 0.0068, 2.1563 ± 0.0047, 1.7481 ± 0.0030, 1.4934 ± 0.0052 and 1.3792 ± 0.0015 µg GAE/g, respectively.

TABLE 5. Total phenolic content of *M. assamica* extracts

Extract	Total phenolic (µg GAE/g)
M. assamica leaf hexane extract	1.3792 ± 0.0015
M. assamica leaf ethyl acetate extract	1.4934 ± 0.0052
M. assamica leaf methanol extract	3.0028 ± 0.0068
M. assamica bark hexane extract	1.7481 ± 0.0030
M. assamica bark ethyl acetate extract	2.1563 ± 0.0047
M. assamica bark methanol extract	3.1170 ± 0.0166

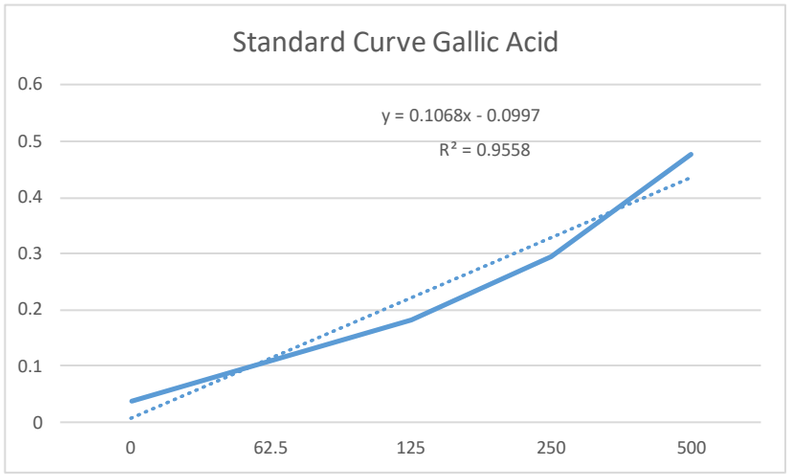

FIGURE 2. Gallic acid calibration curve

Total flavonoid content of M. assamica extracts

The flavonoids content was expressed as µg Quercetin equivalence (QE) per gram of extract by reference to standard curve (Figure 3). Based on total flavonoid content shown in Table 6, *M. assamica* bark hexane extract exhibited the highest total flavonoid content of 31.1484 ± 0.03 µg QE/g, followed by bark ethyl acetate, bark methanol, leaf ethyl acetate, leaf hexane, and leaf methanol extracts which were 31.02765 ± 0.01, 30.95138 ± 0.02, 27.2907 ± 0.05, 14.8026 ± 0.19 and 7.9739 ± 0.15 µg QE/g, respectively.

TABLE 6. Total flavonoid content of *M. assamica* extracts

Extract	Total flavonoid (µg QE/g)
M. assamica leaf hexane extract	27.2907 ± 0.05
M. assamica leaf ethyl acetate extract	14.8026 ± 0.19
M. assamica leaf methanol extract	7.9739 ± 0.15
M. assamica bark hexane extract	31.1484 ± 0.03
M. assamica bark ethyl acetate extract	30.95138 ± 0.02
M. assamica bark methanol extract	31.02765 ± 0.01

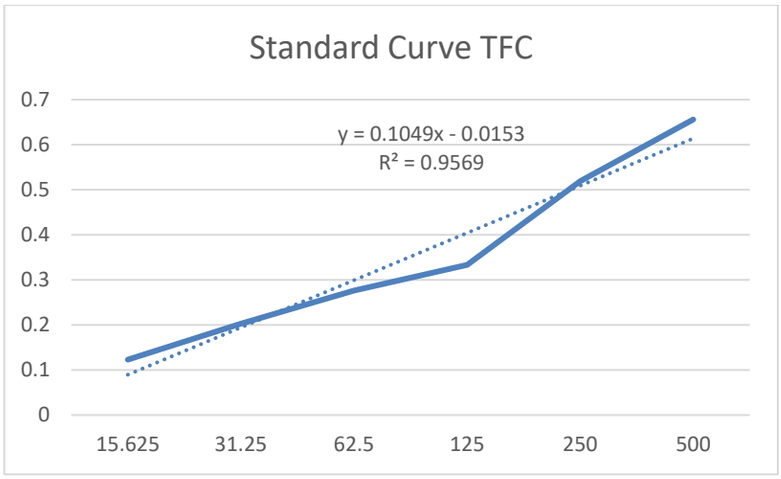

FIGURE 3. Quercetin calibration curve

Free radical scavenging activity of extracts: DPPH assay
The results of DPPH free radical scavenging activity on leaf and bark extracts of *M. assamica* are summarized in Table 7. The standards used for the DPPH assay in this study were ascorbic acid with inhibitory concentration (IC_{50}) value of 11.25 µg/mL. As indicated in this Table 7, bark methanol and leaf methanol extracts of *M. assamica* showed the highest antioxidant capacity among all the extracts tested, with inhibitory concentration (IC_{50}) values of 26.71 µg/mL and 67.11 µg/mL, respectively. Furthermore, significant antioxidant activity in the hexane, ethyl acetate and methanol, extract indicated that the selective extraction of antioxidants by appropriate solvent is very important in obtaining fractions with high antioxidant activity, such as phenolic compounds (TPC). Flavonoids and phenolic compounds have redox properties which allow them to act as antioxidants.

TABLE 7. Antioxidant activity of *M. assamica* extracts using DPPH method

Samples	IC_{50} (µg/mL)
M. assamica bark hexane extract	374.85
M. assamica bark ethyl acetate extract	201.80
M. assamica bark methanol extract	26.71
M. assamica leaf hexane extract	199.34
M. assamica leaf ethyl acetate extract	91.96
M. assamica leaf methanol extract	67.11

Antiproliferative activity assay of the extracts
Table 8 highlighted the IC_{50} values of human breast cell line (MCF-7) and human adipocyte cell line (3T3) treated with crude extracts of *M. assamica.* From this screening, the hexane extract of *M. assamica* bark and leaf parts demonstrated strong cytotoxic activity against MCF-7 with IC_{50} value of 1.97 µg/mL and 7.05 µg/mL, respectively. In addition, ethyl acetate extracts of *M. assamica* leaf exhibited strong cytotoxic activity against MCF-7 with IC_{50} value of 6.33 µg/mL. On the other hand, bark ethyl acetate extract and all methanol extracts demonstrated moderate to weak activity towards MCF-7. However, all extracts were considered inactive towards proliferation of normal human adipocyte cells (3T3).

TABLE 8. MTT assay results of *M. assamica* extracts against human breast cancer cells (MCF 7) and human adipocyte cells (3T3)

Extract	IC_{50} (μg/mL)	
	Human breast cancer cells (MCF-7)	Human adipocyte cells (3T3)
M. assamica bark hexane extract	1.97	32.67
M. assamica bark ethyl acetate extract	29.25	215
M. assamica bark methanol extract	94.68	141
M. assamica leaf hexane extract	7.05	31.85
M. assamica leaf ethyl acetate extract	6.33	53.02
M. assamica leaf methanol extract	36.45	30.59

DISCUSSION

Before the advent of orthodox medicine, for centuries, medicinal herbs have been employed. Herbal medicines can contain leaves, stems, flowers, seeds, fruit, roots and bark, among other things. These plants' therapeutic capabilities are due to their phytochemical constituents, which have defined physiological effects on the human body (Akinmoladun et al. 2007). Isolation of pure, pharmacologically active components from plants is still a time consuming and tedious process. As a result, methods that remove unnecessary separation procedures must be available. As a result, chemical screening is used to enable the identification and targeted isolation of new or valuable constituents with prospective activities. This procedure, which allows the identification of recognized metabolites in extracts or at the early stages of separation, is thus economically critical (Peteros & Uy 2010). The qualitative phytochemical screening of *M. assamica* leaf and bark extracts showed the presence of anthraquinones, flavonoids, alkaloids, cardiac glycoside, reducing sugar, steroids, triterpenes, lipids, phenols, coumarins, carbohydrates, proteins, and betacyanin.

Flavonoids, often known as nature's tender pharmaceuticals, are a vast collection of naturally occurring plant phenolic compounds that include flavones, flavonols, flavonones, chalcones, and isoflavones, as well as having a wide range of biological and pharmacological properties. Flavonoids anti-fungal, antiviral, antioxidant, antiallergenic, anticarcenogenic, hepatoprotective, antithrombic, and cytotoxic have sparked interest in flavonoid-containing plant research. Coumarins have long been acknowledged to have antioxidant, anti-inflammatory, hepatoprotective, anti-allergic, antiviral, antithrombotic, and anticarcinogenic properties. They are phenolic compounds made up of fused benzene and pyrone rings. Hydroxycoumarins are efficient metal chelators and free radical scavengers since they are typical phenolic compounds (Kostova 2005).

The presence of these important metabolites indicated that the use of *M. assamica* plant in various diseases treatment by local or herbalist. These secondary metabolites particularly flavonoids, steroids, anthraquinones, and coumarins possess various pharmacological importance. The plethora of active ingredients endows the plant with exceptional pharmacological qualities, which may explain its wide range of therapeutic purposes. From this study, the qualitative phytochemical screening results of *M. assamica* extracts are in accordance with the results of Phukan et al. (2017).

The total phenolic content of all crude extracts was measured using the Folin-Ciocalteu method. Based on total phenolic content results, methanolic leaf extract of *M. assamica* presented the highest total phenolic contents through the extracts with value of 3.11 μg GAE/g indicating the higher solubility of the

phenolic compounds in methanol. The bark methanol, bark hexane, bark ethyl acetate, leaf ethyl acetate, and leaf hexane extracts possessed high to moderate phenolic contents ranging from 1.37 to 3.11 µg GAE/g. Secondary metabolites called phenolic compounds can donate hydrogen, quench singlet oxygen, and function as metal chelators, making them antioxidants (Michalak 2006). Flavonoids are a broad group of polyphenolic compounds with benzopyrone structure that benefit that produces them in multiple of ways (Kumar et al. 2010).

On the other hand, the highest total flavonoid in this study was found in *M. assamica* leaf hexane extract with flavonoid content of 7.9739 to 31.14 µgQE/g. Flavonoids have been found to be very useful as an antimicrobial agent, an antiulcer agent, a mitochondrial adhesion inhibitor, an antiangiogenic agent, an antiarthritic agent, and an anticancer agent (Biju et al. 2014). In the current study, the presence of flavonoid and phenolic components in *M. assamica* leaf and bark substantiate the health advantages connected with it.

In the present study, DPPH assay was applied as it is a very popular spectro-photometric method to determine antioxidant activity, which involved the measurement of color disappearance caused by free radicals. To form a stable molecule, DPPH absorbs an electron or hydrogen radical as a free radical. The ability of DPPH radicals was determined by the decrease in its absorbance at 517 nm caused by antioxidants. Due to the abstraction of hydrogen atoms from antioxidant compounds, DPPH was reduced to pale yellow colour. The greater the antioxidants exist in the extract, the greater the reduction of DPPH would exist. The elevated scavenging activity by certain sample is closely related to the mitigation of DPPH. IC_{50} refers to the quantity of antioxidants present in a sample that is required to reduce the initial DPPH concentration by 50%. The higher the antioxidant activity, the lower the IC_{50} value (Basma et al. 2011). The results of scavenging activity testing showed that *M. assamica* bark and leaf methanol extract had the highest antioxidant capacity of all the extracts tested, with an inhibitory concentration (IC_{50}) of 26.71 µg/mL 67.11µg/mL, respectively. This suggests that methanol extracts of *M. assamica* contain chemicals that can donate hydrogen to a free radical in order to remove the odd electron that causes the radical's reactivity. Variances in antioxidant activity across extracts from different regions of the plant could be ascribed to chemical composition differences. Secondary metabolites are really stored in many parts of plants, and their concentration in each part fluctuates depending on the plant's exposure to the environment (Ghasemzadeh et al. 2016). Good correlation was found between IC_{50} DPPH values in *M. assamica* leaf and bark of methanol extracts and phenolic contents. This shows that the phenolic chemicals in the plant may be responsible for the antioxidant action of the plant extracts. Furthermore, the flavonoids content of the various component extracts and their IC_{50} DPPH values showed a moderate connection. This suggests that flavonoids, which are subgroups of phenolic compounds, have lower antioxidant activity than phenolic compounds, which are the primary antioxidant contributors in plants (Basma et al. 2011). As a result, phenolic substances other than flavonoids may be responsible for the antioxidant action of *M. assamica* leaf and bark.

Plants have a nearly limitless ability to generate chemicals, which attracts researchers looking for new and innovative chemotherapeutics. The ongoing hunt for novel anticancer chemicals in medicinal plants is a viable and promising cancer prevention technique (Vijayarathna & Sasidharan 2012). Therefore, in this study, the cytotoxicity was evaluated on *M. assamica* leaf and bark extracts by using MTT assay. Two cell lines were chosen to better comprehend the cytotoxicity impact of *M. assamica* extracts on cancer cells; the human breast cancer cell line, MCF-7 with reference to the normal human adipocyte cell line, 3T3. From this screening, hexane extract of *M. assamica* bark and leaf parts as well as ethyl acetate extract of *M. assamica* leaf demonstrated potent cytotoxic activity against MCF-7 with IC_{50} value of 1.97 µg/mL, 7.05 µg/mL and 6.33 µg/mL particularly. On the other hand, bark ethyl acetate extract and all methanol extracts displayed moderate to weak activity towards MCF-7. According to Vijayarathna and Sasidharan (2012), The American National Cancer Institute has mentioned that a crude extract can be treated as active or highly cytotoxic with IC_{50} value less than 20 µg/mL. Hence, all extracts were considered inactive towards proliferation of normal human adipocyte cells (3T3) with IC_{50} value of more than 30 µg/mL. This study suggests that non-polar extracts of *M. assamica* bark and leaf exhibited good cytotoxic activity and could be useful in the creation of cancer-fighting drugs.

Conclusions

Phytochemical screening of medicinal plants is critical for discovering new sources of therapeutically important compounds. The results gathered in this study indicated the presence of a diverse group of phytochemicals from *M. assamica* extracts. The antioxidant and

antiproliferative characteristics of *M. assamica* extracts used in this study are due to these phytochemicals, which are known to support bioactive activities in medicinal plants. The antioxidant potential of *M. assamica* bark and leaf methanol extracts was found to be the highest of all the extracts tested, with inhibitory concentrations (IC_{50}) of 26.71 g/mL and 67.11 g/mL, respectively. Also, the study clearly indicates that hexane extracts for bark and leaf (1.97 and 7.05 µg/mL) and ethyl acetate extract (6.33 µg/mL) showed good cytotoxicity activity. These phytochemical and cytotoxicity studies that showed the cytotoxic properties of *M. assamica* extracts suggest them to be a persuasive resource of natural chemopreventive agents in drug discovery. From this study, the active crude extracts of *M. assamica* were exposed to several pharmacological assessments by numerous methods for the extraction and identification of bioactive components, which could provide a better source for generating new therapeutic medicines.

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REFERENCES

Adhikari, P., Bhatia, H., Khatri, D.B., Srivastava, G., Uhl, D., Mehrotra, R.C. & Paudayal, K.N. 2022. Plant fossils from the middle Siwalik of eastern Nepal and their climatic and phytogeographic significance. *Palaeobiodiversity and Palaeoenvironments* https://doi.org/10.1007/s12549-022-00523-5

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. & Farombi, E.O. 2007. Phytochemical constituent and antioxidant activity ofextract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essay* 2(5): 163-166.

Ayoola, G.A., Coker, H.A., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. & Atangbayila, T.O. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research* 7: 1019-1024.

Basma, A.A., Zakaria, Z., Latha, L.Y. & Sasidharan, S. 2011. Antioxidant activity & phytochemical screening of the methanol extracts of *Euphorbis hirta* L. *Asia Pacific Journal of Tropical Medicine* 4(5): 386-390.

Baruah, P.S., Borthakur, S.K. & Tanti, B. 2021. Notes on lectotypification of the Assam Ironwood *Mesua assamica* (King & Prain) Kosterm. (Calophyllaceae). *Journal of Threatened Taxa* 13(8): 19181-19184.

Biju, J., Sulaiman, C.T., Satheesh, G. & Reddy, V.R.K. 2014. Total phenolics an flavonoids in selected medicinal plants from Kerala. *International Journal of Pharmaceutical Sciences* 6: 406-408.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol.* 28(1): 25-30.

Chang, Y.P., Mazurah, M.P. & Nik, M.M. 2008. *Plant Health and Man-Past, Present and Future*. Selangor: Forest Research Institute. p. 342.

De, S., Dey, Y.N. & Ghosh, A.K. 2010. Phytochemical investigation and chromatographic evaluation of the different extracts of tubers of *Amorphaphallus paeonifoleus* (*Araceae*). *International Journal on Pharmaceutical and Biomedical Research* 5: 150-157.

Dutta, M.P., Singh, M.K. & Borah, D. 2020. Piscicidal plants of Northeast India and its future prospect in aquaculture-A comprehensive review. *Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR*)] 10(3): 165-174.

Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. 2005. Phytochemical contituents of some Nigerian medical plants. *African Journal of Biotechnology* 4(7): 685-688.

Ee, G.C.L., Teh, S.S., Kwong, H.C., Mah, S.H., Lim, Y.M. & Rahmani, M. 2012. A new benzophenone from *Mesua congestiflora,* an inhibitor against human B lymphocyte cancer cell line. *Phytochemistry Letters* 5(3): 545-548.

Ghasemzadeh, A., Jaafar, H.Z., Ashkani, S., Rahmat, A., Juraimi, A.S., Puteh, A. & MudaMohamed, M.T. 2016. Variation in secondary metabolite production as well as antioxidant and antibacterial activities of *Zingiber zerumbet* (L.) at different stages of growth. *BMC Complementary and Alternative Medicine* 16(1): 104.

Gogoi, B. 2017. Anti-cancerous potentiality of coumarins of *Mesua assamica* (King &Prain) Kosterm–An endemic plant of assam. *International Journal of Pharmacognosy and Phytochemical Research* 9(7): 939-942.

Harbone, J.B. 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. UK: Chapman & Hall. pp. 2-3.

Handore, A., Khandelwal, S. & Bholay, A. 2018. *V. vinefera*: A promising source of resveratrol for nutraceutical applications. *National Conference on Innovations in Food, Environment and Healthcare (NCIFEH-2018),* September 28-29, p. 7. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*. DOI 10.26479/2018.0406.40

Ibrahim, J. 2004. Medicinal Plant Research in Malaysia: Scientific Interests and Advances. *Jurnal Sains Kesihatan Malaysia* 2(2): 27-46.

Kostova, I. 2005. Synthetic and natural coumarins as cytotoxic agents. *Current Medicinal Chemistry - Anti-Cancer Agents* 5(1): 29-46.

Kumar, P.P., Kumaravel, S. & Lalitha, C. 2010. Screening of antioxidantactivity, total phenolics and GC–MS study of *Vitex negundo. African Journal of Biochemistry Research* 4: 191-195.

Michalak, A. 2006. Phenolic compounds and their antioxidant activityin plants growing under heavy metal stress. *Polish Journal of Environmental Studies* 15: 523-530.

Phukan, H., Bora, C.R. & Mitra, P.K. 2017. Phytochemical screening and gc-ms analysis of methanolic leaf extract of an endemic plant *Kayea assamica*. *Journal of Pharmacy and Biological Sciences* 12(5): 7-16.

Peteros, N.P. & Uy, M.M. 2010. Antioxidant and cytotoxic activities and phytochemicalscreening of four Philippine medicinal plants. *Journal of Medicinal Plants Research* 4(5): 407-414.

Satheesh, K.B., Sharmila, K.P., Suchetha, K.N. & Vadisha, B.S. 2013. Acute and subacute toxicity study of the ethanol extracts of *Punica granatum* (linn). Whole fruit and seeds and synthetic allergic acid in swiss albino mice. *Asian Journal of Pharmaceutical and Clinical Research* 6(4): 192-198.

Shekhar, T.C. & Goyal Anju, G. 2014. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* linn. leaves. *American Journal of Ethnomedicine* 1(4): 244-249.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H. 2011. Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia* 1(1): 98-106.

Vijayarathna, S. & Sasidharan, S. 2012. Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and vero cell lines. *Asia Pacific Journal of Tropical Medicine* 2(10): 826-829.

Wiratchanee, M., Vithoon, V., Wanna, C., Arunporn, I. & Kesara, N.B. 2010. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells *in vitro*. *BMC Complementary and Alternative Medicine* 10(55): 1-8.

Yang, B., Yang, H., Li, J., Li, Z. & Jiang, Y. 2011. Amino acid composition, molecular weight distribution and antioxidant activity of protein hydrolysates of soy sauce lees. *Food Chemistry* 124(2): 551-555.

*Corresponding author; email: hazlinaahmad@nuclearmalaysia.gov.my